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Liew, Choong-Chin

<120> Method for the Detection of Gene Transcripts in Blood and Uses  
Thereof

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<150> US

<151> 2000-01-04

<150> US 60/115,125

<151> 1999-01-06

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<170> PatentIn version 3.2

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tatcaaatta taaaccacc cacattactt aaaattttt acatttccca ataaaaaacc 120

45 caaataaaca aaaacttcca atctccattt aaat 155

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25 gaaaaanttt tccggggggc ccacccggga agggntnccg gggaaggg 168

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cccacattc cattttaat atatactgtg ctttaccat attataatat gttttaaaat 180  
40 atgttcacag aagcacctgg tctgtgaatg gcatgccagc attaaaaaa ataagcattc 240  
tttgaatata tatttagttt ttaattgtg taggaaaatc aaagccagag ggagtagaaa 300  
caaaattgt gattttctaa atacttctg gctgcaggga agaaaccacg tcccaggcga 360  
45 agtcctacct aattgatga taaaattaca tggaagggat tctgttggc atgaggacct 420

accaagatgg tcaacaga

438

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15 aaagggggaa ggcccccaa accggggggg gggnaaaaag gtgggtttcc cccttttcc 180  
  
aattcccaa accaatttc aaaaggnaaa ccaacnttc caaaatggg aaagg 235

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 45 caaattgggg ggnaaaccaa atttaagggg ggaagggggg gnccccccg ggaaaggccc 180



aaggggggaa aatttttccg ggggtgggtg gggggaacca atttaagggg ggggcccccg 240

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15

ctgtacttga actctaaac tgttgagaa actcagtgt taccccaaca gattcattc 180

aaatagctgt aaaaggtatg ttactccag aagaccagag ttgcttctt tgaacttctc 240

20

attccttggg cctaggaacc ctcatcacc tcacccaac gtcaaccag atcttctctt 300

ccataaacag cactccctca ggcccctgcc tgacacaggc atagactgtc atgttgatt 360

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tttagcggc accccaacaa caccaggccc tactttttcc aaggncgggg aagcccatgg 180  
45 gttctgggna acgggcaatg cgggcttgca acgggnggaa naaaacagn cccaaaagaa 240

tg 242

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 tctcagctca ctgcaacctc cacctctcag gagaattgct gaacctggga ggcggaggtt 120  
 15 gcagggagct gagattgcgc cactgccctc catcctgggc gacagagcaa gaacctgtct 180

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 15 aaggggggtg gggaagagcc aaatttctt gggaanaac gcccccttgg ggaaaanaag 180  
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 30 ggaagaattg tatgtgggag ttttaatgg tctcatttca ttggctataa ctgagttaca 180  
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      ttgaggcgaa aggactcgcg gatttgacgc ttgatgcggt tgcgctcgac ggcgagcttg    180  
      acgctctttt tgccgatcac caaacctagg cggggatgat caagctgggt atcgcgcgct    240  
      agcagcagga cacttttgcc cgggagcttt accgcttggg gagtcgaaga ctgccttgna    300  
      ttgccgggga gtcagcagtc gcttttccc ggncgaagcc tcgaactcac cancctgtct    360

ggattaatta gacagcaaga cgcttgccgc ccctttggcg cgaacgaacn ncgaaaagga 420  
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 5 tgggcgccag caggggcggc ctccgggcca aaccgaacct caggtcanac ttccaagaaa 360  
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aagaaggaac ccacctngg ggccaaacaa aaacttaaaa acccccccat ttctncccc 180  
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 cctgtgaaac ccataaacag tgggaaatgg tgc 153  
  
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30 ggtgctcctg cgtttggact gtgcaggcct ctctatgct gatgcgtctc cccactcctg 180  
  
agctaatttc tgtctgctc ctctgtgac atgtggcagc gtgggaaata gccactgtcc 240  
  
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5 actagatttt actgtagatg gtagataaaa gtccagtgaa aagcaaagat gtgtaatgtt 180

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gaagagtctt agtgcattta ttggccag aaccctctaa tccccttgta atttatgtca 240

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tatatctcaa atgtcttgc aacagtgggt actttgctct atccttagct atacgtctcg 180

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45 ctgattaga 309

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 10 gatgacataa aatgagatga aatgagatgt aatgatggaa tgagatgaga tgaaatgaga 120  
 tgaaatgata gatgagataa aatgatgata tgaaatgatg agatgaatga tgagatgatg 180  
 15 agatgaatga tgaaatgaaa tgatgagatg agatgatgaa atgaaatggt gagatgaaat 240  
 gatgagatga aatgaaatag tgaaatgaaa ttgaaataaa atcgaaatga gagatgaaat 300  
 gatgagatga tgaaattgat gaaatgatga gatgtgatga gatgaaatga tgagatgaga 360  
 20 tgagatgaca tgaaataatg aatgaaatt gaaatgagat aagatacgag ctgagatgca 420  
 atgagatgaa atgatgagat gaaatgaaat agtgaaatga aattgaaata aaatcgaaat 480  
 25 g 481

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<223> n is a, c, g, or t

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 15 ngacgcgccg tacaggntna attatggan cttacatggn aaaggggcan ctcaatgtcc 120  
  
 cacag 125  
  
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 atgagatgaa atgttgaaaa gaaaggagga aatgatgagg tgagatgaaa tgatgagatg 120  
 35 aatgaatct gagatgaaat gagatgaaaa ntgatacgaa aaatgatata aaaaatatga 180  
 cctgagatga aatgagatga aaaatgatac gaaaaatgat ataaaaaata tgacatgaaa 240  
 40 tgaaatgaga tgatatgaaa tgacataatg aaatgatgaa ttgatgatat tgaaatgaaa 300  
 ttgaaagatg agatgaaatg atgagatgaa atgaaatgtt gaaatgatga agagatgtga 360  
 catgaaatga gctgaaatga gatgaaatga aatgagatta aatgatgaga tgaaaaatga 420  
 45 tgagatgaaa aatgagatga gatgatgaga tgagatgaga tgaattgaga tga 473

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 <223> n is a, c, g, or t

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 aaatgaaatt agatgaaatg taatgagatg aaatgaaatg acctaataa atgaaataat 120  
 gaaatgagat gaaataaaat aatgaaatga tgaaataatg aaatgaaaat gagatggaaa 180  
 30 tgatgagatg agaagaaatg atgagatgaa atgatgaaat gatgagatga ganaaaatga 240  
 gatgaaatga tgagatgaga tgaaatatga tgagttgaaa tgacataatg aatgaaatga 300  
 35 tgaaatggaa taatgaaatg gaaatgatga gctgagatgc aatgagttga aatgagatga 360  
 aatgatgaaa tgatgagatg aaatgatgaa atgaaataat gaaatgagat gaaataaaat 420  
 aatgaaatga tgaaataatg aaatgaaaat gaaatggaaa tgatgagatg agaagaaatg 480  
 40 atgagatgaa atgatgaaat 500

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<223> n is a, c, g, or t

<400> 74

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tgaaatgatg agatgaaatg atgagatgag atgtgatgaa atgatgatat gaaatgatga 120

cataaaatga gatgaaatga gatgtaatga tggaatgaga tgagatgaaa tgagatgaaa 180

20 tgatagatga gataaaatga tgatatgaaa tgatgagatg aatgatgaga tgatgagatg 240

aatgatgaaa tgaaatgatg agatgagatg atgaaatgaa atggtgagat gaaatgatg 299

25

<210> 75

<211> 155

<212> DNA

<213> Human

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<400> 75

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taaaatgatg aaatgatgag gtgatgagat gaaatgatga gatgaaatga tgagatgaga 120

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40 <211> 367

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 cgccatgaaa ttctgctcg attagcttac gttgtttgga tagaggccaa acaaggctgt 180  
 45 tatcggtagc aggaatggat gttcgatttc gtagaatagc cctgagagac ggcaataact 240

ctcacgagag gcagcaggcg cgtaaattac ccaattacaa caagtagagg tagcgaagga 300

aaatatgagg ggtggcaagg tttgcctgt tacattctca aatggaagca aattagatat 360

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gtcattg 367

<210> 77

10 <211> 257

<212> DNA

<213> Human

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<223> n is a, c, g, or t

20 <220>

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<223> n is a, c, g, or t

25 <400> 77

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atagaaatca ttatttctgt gggatggata attgggccaa attgtaaaat attttaacta 120

30 tcagtgtttg gggtttattt taaaagaat aggggtgccac cagatgttct ttagtggagg 180

agaaatgagg ccagagtgcac tgcctagaaa attaagttgg taaattaatc actttttct 240

aggtcctttc ttagtct 257

35

<210> 78

<211> 373

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40 <213> Human

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 nntaantaaa aagggtgaag aannttactt cncctgggcc taaaaacnt ttcntcagt 120  
 30 tattacaaa atattggac cattantaaa gantagggcc aaccnaatt ttcttgaaa 180  
 ttccgtaa atagccgtta aatgtttta cccattcat attggatacc ttaaattata 240  
 35 ataatggatt ttattgttaa attgtgtgtg tgtgggtgtg atgccctgtc tttctcctc 300  
 taccattatt gtcactttat gtttgaacc ccctttaccc ttccttaaag gaaaaaaagg 360  
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 35 ccttgatggt caagtattta agaccctatg cnaatatttt ttaccttttc taataaacca 120  
  
 tgtttggt 128  
  
 40 <210> 80  
 <211> 213  
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 45 <220>



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<223> n is a, c, g, or t

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ggccaggcct tcctttaa aaaaaatnac cgttttngg gggangnaac ctttaaagg 120

ggaaaanaaa tccttttaa anggaantcc aagggaagga ncctgncaa nactcccn 180

30 ccaataaaaa aaacntttt ggaaangggg aaa 213

<210> 81

35 <211> 443  
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45 <221> misc\_feature

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 <223> n is a, c, g, or t  
  
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 tgatgaaatg agatgagatg agaagaaatg acttgatgag atgagataaa atgatgaaat 120  
  
 gaaatgaagt gaaatgaaat tgaaatgaga tgagatgaaa tgagataaaa tgatgagatg 180  
 15 aaatgagaag aaatgagatg aaatgatgaa atgatgagat gagatgaaaa atgatgggat 240  
  
 gagaaatgag atgaaatgat gggatgaaat gaaatgaaat aatgaaataa tgaaatgaaa 300  
  
 20 tgaattgata atattgaagt gaaattgaaa gatgagattg gatgaaatga tgagatgaaa 360  
  
 tgaaatgttg aaatgaaatg aagagatgta acatgaaatg agctgaaatg atgagatgaa 420  
  
 atgaaatgaa atgagattaa atg 443  
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 15 <223> n is a, c, g, or t  
  
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 20 <223> n is a, c, g, or t  
  
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 25 gacctctgaa tgtattantg anncaagtga tagccaagat attttgaaga aaaatagata 120  
 ntagggacct gctctataag cccatcataa ttattatga agttataaca agtaaacag 180  
 taaggtattt ggcatggaat agagaaccca gaaacagacc caatgcatgg gtacaggata 240  
 30 taacacaggg aaatgagggg caatatatgg ttctgggata attatttata tggggaaaat 300  
 aaagaaattg gatccctacc tcacacatac aaaaaaaatc ataattgaat taaaaacttg 360  
 35 catgtgaaag gaaagacttt aaaacattta gaaaaagtat tggaggctat gatcttgggg 420  
 taggaaagca ttctttttt tt 442  
  
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5 <220>  
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<223> n is a, c, g, or t

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aggagggaaa agaaagaaag gaaggaagga aggaaaagaa agaaagaaag gaaggaagga 120  
  
15 aggaaggaag gaagg 135

<210> 84  
<211> 346  
20 <212> DNA  
<213> Human

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gagttgcctt ctgtaagtgc ccctactatc aaggttcat ggaaaatcta ggcaaggcag 120  
  
aacttcctca gaaggacaag agacaaagaa gtgggggagg ccctcctatc catagctgag 180  
35 agggtttatt ctttgtggtt ctgctgtcag agcctttgga tgtctgatct gagatggagc 240  
  
aaccacagct agacagaact ttgtagattt tgggggggtt aaaaggcctc aagcaaattc 300  
  
40 taaaacttc ttgaacccc ctggcatagg ctcagttcc ctgact 346

<210> 85  
<211> 100  
45 <212> DNA  
<213> Human

<400> 85  
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5 gaaaagaaaa tggtttttt tgccgaaatc aaccgggtaa 100

<210> 86  
<211> 201  
10 <212> DNA  
<213> Human

<400> 86  
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15 gaaaagagga agaaacgctt ttaggaggtt aaggattaaa gtaaaaatta tttgacata 120

attacctctt tttgtacca ctctaaagg ccaggaacat atttgagaa gcctagtgt 180

20 atgtaacagt gtggggttc a 201

<210> 87  
<211> 531  
25 <212> DNA  
<213> Human

<400> 87  
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30 aaccagaac gaacgcgtta agtgcgcgtt atcttctag gatagagtcg gtgacgggaa 120

tctttaccc cggcactcgg gtccaccctc gcggcaccag aggtattctc cggcgagtcg 180

35 ttaaccatcg caatgccga ccgagtttaa ggaccactcc ccaccttct cattagtaa 240

ggagaacgct actttacccc atagacggag aaatcgctac tcaactacca ggcgcgcgcc 300

gtcgagtccc tcttctctc ttatgcatt tagagcgctt tcgtaagagt tttccctaga 360

40 ttttctaag cgtagcgcgt ctactccaat gtttcgtta atccagcccg aactaacgcc 420

gcggaggagt cgatccgtct actcctatcc cgtcggctcg gatttactac aggagctaag 480

45 aaaacaaaa gtaccagccc taaaggaaag tcaaaggacg cccgtaaaaa a 531

<210> 88  
 <211> 530  
 <212> DNA  
 5 <213> Human  
  
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 10 cattcgggtcc agtgcctcgc cctattagcc cttagccca ccgtttctaa aactccaga 120  
 acagccaaac cgggtccgcc aaggcctccg tcgtttata atatattccg ttacgtata 180  
 aggaacgaac ccccttcat taccacggtc ccgcgtccgc ctcttctcc attcgcaaca 240  
 15 gttctattcc tttagcctc ccgtacctgc ttccagaaca tcgcaccgcc atagtcgaaa 300  
 gatagcaaag attaccagc ttctattcct cgccccagag ccgagtaaatt cgaagtttat 360  
 20 agaggcggaa tccaaccatt caagagttat aacaagttat cggcactcgg gggatcagaa 420  
 tataaactta atgtcccctt tattctcccg gacgcccctt ttaaccatt ctctctatct 480  
 ttcgctaaca agccattgac ggcgctttgc cgcgcggggc catctcgcgt 530  
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<210> 89  
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 30 <213> Human

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<223> n is a, c, g, or t

<220>

5 <221> misc\_feature  
<222> (763)..(763)  
<223> n is a, c, g, or t

<220>

10 <221> misc\_feature  
<222> (778)..(778)  
<223> n is a, c, g, or t

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15 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

20 <221> misc\_feature  
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

<400> 96

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aaatgaaatg aaataatgaa atgaaattga aataaaattg aaatgagatg agatgaaatg 120

atgagatgat gaaataaaat gatgaaatga gatgtgatga gatgaaatga ngagatgaaa 180

40 tgatgagatg agatgacatg aaataaatga aataatgaaa tcgaaatgag atgagaagat 240

acgagatgag atgaaatgat gagatgaaat gatgaaatga gataagatga aaagagttga 300

45 tgagatgatg agatgaaatg agatgaaaag agatgaaatg agatgaaatg aaatgatgag 360

atgaaatgag gtgaaatgaa attagatgaa acgtaatgag atgaaatgac ataatgaaat 420  
 gaaaaaatga aatgaaataa tgaaatgagg tgaaattaaa tgagatgatg aaattaaatg 480  
 5 atgaaatgaa ataatgaaat ggaaatgaaa tggaaatgat gagatgaatg atgagatgaa 540  
 atgatgagat gagatgtatt gatgagagga aatgatgaga tgtaatgaaa tgagatgaaa 600  
 tgaatgagat gaaatggaat antggaangg aaattgattg gngatttgag atgaaatgag 660  
 10 ntaaatngna tgaattaatg atgagatgaa atgntgaatg ccgggggtggn tgagatgaat 720  
 tgagttgaac cctgngatga atgaagattg nntgaatggt ggntgaatgt tgaatggntg 780  
 15 gntggnanaa tgcctgtngg 800

<210> 97  
 <211> 334  
 20 <212> DNA  
 <213> Human

<400> 97  
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 gaaatgaaat gatgaaatga atagatgtga catgaaatga gctgaaatga tgagatcaaa 180  
 30 tgaaatgaaa tgagattaaa tgatgagatg aaaactgatg aaaacttaaa tgatgaaata 240  
 atgaaatgaa aatgaaatgg aatgatgag atgagaagaa atgatgagat gagatgagat 300  
 aaaatgagat gaaatgatga gatgaaatga tgag 334  
 35

<210> 98  
 <211> 100  
 <212> DNA  
 40 <213> Human

<220>  
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 45 <222> (17)..(17)  
 <223> n is a, c, g, or t

<400> 98

ttcaggccgt ctgcttntac atatactatc gagaatgggtg ctgtgcactc ataaccgt 60

5 tgcttggttag acgcttttga acccttcagc gctgaaagta 100

<210> 99

<211> 500

10 <212> DNA

<213> Human

<220>

15 <221> misc\_feature

<222> (8)..(8)

<223> n is a, c, g, or t

<220>

20 <221> misc\_feature

<222> (361)..(361)

<223> n is a, c, g, or t

<400> 99

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agatgaaaag agttgatgag atgatgagat gaaatgagat gaaaagagat gaaatgagat 120

gaaatgaaat gatgagatga aatgaggtga aatgaaatta gatgaaacgt aatgagatga 180

30 aatgacctaa tgaaatgaaa aatgaaatg aaataatgaa atgaggtgaa attaatgag 240

atgatgaaat taaatgatga aatgaaataa tgaaatggaa atgaaatgga aatgatgaga 300

35 tgaatgatga gatgaaatga tgagatgaga tctaatgatg agaggagatg atgagatgaa 360

ntgagatgaa aagagatgaa atgagatgaa accgaaatga tgagatgaaa tgaggtgaaa 420

tgaaattaga tgaaacgtaa tgagatgaaa tgacataatg aaatgaaaaa atgaaatgaa 480

40 ataatgaaat gaggtgaaat 500

<210> 100

45 <211> 397

<212> DNA

<213> Human

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10 <221> misc\_feature  
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

<220>

20 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

25 <221> misc\_feature  
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<223> n is a, c, g, or t

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30 <221> misc\_feature  
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<223> n is a, c, g, or t

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35 <221> misc\_feature  
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<223> n is a, c, g, or t

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40 <221> misc\_feature  
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<223> n is a, c, g, or t

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tccccttgaa nntctctaaa ccttaacttt ctggccnttt tgtttcagan atgcctaacc 120  
 ctacaggggt cttttgttct ctacgcctaa aaacttaatc tgtttggaac aattccnttt 180  
 5 cctctctgta gaaattgacc tggccatggc tcctgtgaat gatacggttg ctattatccc 240  
 tgaacactgt aaaaatgaac ttgaaacag ttgggtagga cccaaacaga aaatgatgta 300  
 tggcttgga atagttagc tgaacattat gctttaatat ttactggcc attgcagcac 360  
 10 aggtttagaa atttatgttc ggcttttaa agtttta 397

<210> 101  
 15 <211> 132  
 <212> DNA  
 <213> Human

20 <220>  
 <221> misc\_feature  
 <222> (121)..(121)  
 <223> n is a, c, g, or t

25 <400> 101  
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 attaatgta ctactttcaa aagaattaat tgaagaaaga gagatacagg gtatctaggc 120  
 30 ngaggaagac cc 132

<210> 102  
 <211> 246  
 35 <212> DNA  
 <213> Human

<400> 102  
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 40 ggcactta atttataattg atatatataa taaaaaatc aatgcagata ttgatataat 120  
 aaaaatagat aatggtaatc caagcacgat ggtagccatc actctaattg ctttgggggt 180  
 45 aacctataac ttattaagta aagtgccaga atggttctt gacagtatta aaattaaaga 240



aaacag

246

<210> 103

5 <211> 18

<212> DNA

<213> Artificial Sequence

<220>

10 <223> forward primer of exon 1 of insulin gene used for quantitative RT-PCR analysis

<220>

15 <221> primer\_bind

<222> (1)..(18)

<400> 103

gccctctggg gacctgac

18

20

<210> 104

<211> 18

<212> DNA

25 <213> Artificial Sequence

<220>

<223> reverse primer of exons 1 and 2 of insulin gene used for quantitative RT-PCR analysis

30

<220>

<221> primer\_bind

<222> (1)..(18)

35

<400> 104

cccacctgca ggtcctct

18

40 <210> 105

<211> 24

<212> DNA

<213> Artificial Sequence

45 <220>

<223> forward primer of  $\beta$ MyHC gene used for quantitative RT-PCR

analysis

<220>

5 <221> primer\_bind

<222> (1)..(24)

<400> 105

gctggaacgt agagactccc tgct

24

10

<210> 106

<211> 24

<212> DNA

15 <213> Artificial Sequence

<220>

<223> reverse primer of  $\beta$ MyHC gene used for quantitative RT-PCR  
analysis

20

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<222> (1)..(24)

25

<400> 106

ggatccttcc agatcatcca ctg

24

30 <210> 107

<211> 20

<212> DNA

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35 <220>

<223> forward primer of ANF used for quantitative RT-PCR analysis

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40 <221> primer\_bind

<222> (1)..(20)

<400> 107

ggatttcaag aattgctgg

20

45

<210> 108  
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 <212> DNA  
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5

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 <223> reverse primer of ANF used for quantitative RT-PCR analysis

10

<220>  
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<400> 108

15

gcagatcgat cagaggagtc

20

<210> 109  
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 <212> DNA  
 <213> Artificial Sequence

20

<220>  
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25

<220>  
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30

<400> 109  
 ggatgcttca tgtgaacgtg

20

35

<210> 110  
 <211> 19  
 <212> DNA  
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40

<220>  
 <223> reverse primer of APP used for quantitative RT-PCR analysis

45

<220>  
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 <222> (1)..(19)

<400> 110  
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19

5

<210> 111  
<211> 21  
<212> DNA  
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10

<220>  
<223> forward primer of ZFP used for quantitative RT-PCR analysis

15

<220>  
<221> primer\_bind  
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20

<400> 111  
cacargagrc arggtcaacg a

21

25

<210> 112  
<211> 22  
<212> DNA  
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30

<220>  
<223> reverse primer of ZFP used for quantitative RT-PCR analysis

35

<220>  
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<222> (1)..(22)  
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ggattaaaat gaagcaccca ga

22